ASCARIS CANIS (WERNER) AND ASCARIS FELIS (GÖZE).

A TAXONOMIC AND A CYTOLOGICAL COMPARISON.

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Introduction.

Even to-day systematic helminthologists are not of one opinion concerning the taxonomic relation of the *Ascaris* found in the dog to the *Ascaris* found in the cat. Thus Glaue ('08, '09), after a careful study of hundreds of individuals, maintains that they are two distinct and separate species; whereas two other workers, Schöppler and Krüger ('12), declare quite as emphatically that the differences between these two forms are not specific differences, but differences in degree of development only, and believe that these forms are merely varieties of *Ascaris mystax* (Zeder). In 1911 Edwards made a cytological study of *Ascaris felis*. The chromosomes in number and form differed greatly from those described by Marcus ('06) for *Ascaris canis*. Therefore upon a cytological basis, Edwards declared *A. canis* and *A. felis* to be distinct species. Dr. S. I. Kornhauser, while examining the germ cells of the *Ascaris* from the dog in connection with the formation of di-tetrads, was struck by the dissimilarity between his material and that studied by Marcus. On communicating with Dr. Marcus, it was found that he had obtained his material from a great variety of mammals which died at the Munich Zoölogical Gardens. At the suggestion of Dr. Kornhauser, the writer has made a careful taxonomic and cytological comparison of *A. canis* and *A. felis*. Taxonomically he is able to substantiate the work of Glaue; and cytologically, that of Edwards; and to prove that Marcus was not dealing with *A. canis* (Werner).

I here wish to express my thanks to Dr. Kornhauser for his criticism of my work and the preparation of this paper, and to Dr. Chas. Zell, of Chicago, for aid in obtaining material.

Material and Methods.

The material used was obtained from freshly killed dogs and cats. The worms were immediately removed from the intestine, placed in normal salt solution and kept at body temperature until they could be fixed. The ovaries and testes were at once stripped out on a glass plate and fixed. Hermann's fluid and Flemming's fluid (strong) were used to fix the testes, and Petrunkevitch's modification of Gilson's fluid was found to be the most satisfactory fixative for the ovaries. Sections of the testes were made 4 μ to 8 μ in thickness; those of the ovaries, 10 μ to 30 μ in thickness. In general the material was stained by a modified iron-alum-hæmatoxylin method: dilute Delafield's hæmatoxylin being used in the place of $\frac{1}{2}$ per cent. aqueous solution of hæmatoxylin after the mordant. This method gave better contrast between the chromatin matter and the yolk granules than could be obtained by the ordinary Heidenhain method. Orange *G* and Bordeaux Red were used as counter stains.

Taxonomic Comparison of A. canis and A. felis.

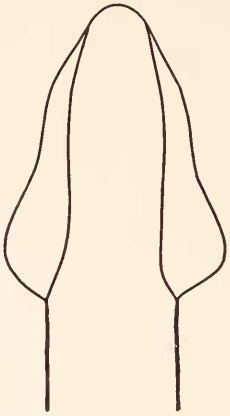
Anatomical study of one hundred specimens of *A. canis* and fifty specimens of *A. felis* gave the results shown in the following table. The table at the same time gives a comparison of the main points of difference between the two forms.

	A. canis.	*A. felis.*
Length of animal	60–120 mm.	45–95 mm.
Length of oral wing	1,950–2,250 mm.	1,450–1,720 mm.
Breadth of oral wing	350–500 mm.	400–600 mm.
Shape of oral wing	Lanceolate	Cordate.
Cross-section, oral wing	Chitinous rod, long and narrow.	Chitinous rod, broad and flat.
Wing spicules	Long, crescent-shaped	Long, semi-circular.
Form of tail of male	Gradual slope to point	Folded ventrad to point.
Postanal papillae	4 ventral, 3 dorsal pairs	3 ventral, 2 dorsal pairs.
Anal spicules	Short, narrow	Longer, broader.
Body segments	Narrow	Broader than *A. canis*.

From this comparison it is seen that *A. canis* is longer than *A. felis* and has an oral wing which is lanceolate rather than cordate in shape (Text-figs. *A* and *B*). Cross-sections of the oral wing show differences in the shape and size of the supporting

chitinous rods of the two forms (Text-figs. *C* and *D*). The number and relative positions of the postanal papillæ in *A. canis*

TEXT-FIG. *A*.



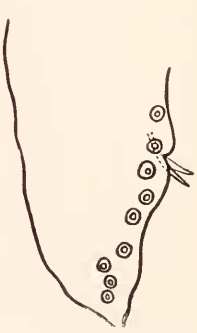
TEXT-FIG. *B*.

differ from those of *A. felis* (Text-figs. *E* and *F*). These results substantiate those recorded by Glaue ('08 and '09), and are of

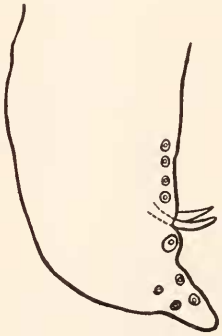
TEXT-FIG. *C*.

TEXT-FIG. *D*.

too constant and specific a character to be classed as variations of the same species, as claimed by Schöppler and Krüger ('12).



TEXT-FIG. *E*.



TEXT-FIG. *F*.

In the specimens examined by the writer there were none of the variations claimed by the writers last mentioned, and therefore the present writer maintains upon a morphological basis that the *Ascaris* of the dog is a species distinct from the *Ascaris* of the cat.

Spermatogenesis of A. canis and A. felis.

In the present article only very characteristic stages in the spermatogenesis will be considered.

1. *A. canis.*

The chromatin of the spermatocytes nearing the end of the growth period appears in the form of two irregular masses situated peripherally in the nucleus. One mass is small and circular, whereas the other is long and narrow, lying within the nuclear membrane and extending along half of its circumference (Plate I., Fig. 1). There is also a plasmosome which loses its affinity for stains and disappears shortly after the formation of the prophase chromosomes. The two chromatin masses early assume a vacuolated appearance and become distinct chromosomes. From the larger chromatic mass are formed twelve large chromosomes; and from the smaller karyosome are formed six small chromosomes, closely grouped. Thus we find early that the haploid number of chromosomes, eighteen, clearly defines itself. The group of twelve large chromosomes retains a peripheral position during the prophase, while the smaller group migrates early to the central part of the nucleus.

The centrosome is of extranuclear origin as it sets up the mitotic figure before the nuclear membrane disappears (Plate I., Fig. 2). The spindle fibers seem to arise from the centrosome and push against the nuclear membrane, which often shows irregular indentations in the region of the centrosome.

In the metaphase of the first spermatocyte division the chromosomes are arranged in the equatorial plate, with the group of six chromosomes in the middle and the twelve larger chromosomes scattered more peripherally (Plate I., Fig. 3). From a polar view these peripheral chromosomes all show a distinct tetrad form. From a lateral view, careful study reveals that the twelve large chromosomes also show a tetrad form (Plate I.,

Fig. 4), indicating that the chromosomes of the first maturation spindle are really di-tetrads, or octads, due to the presence of a "Querkerbe" in each of the four components. The number of chromosomes is easily seen to be eighteen (Plate I., Fig. 3).

The anaphase of the first spermatocyte division shows that the group of six small chromosomes lags behind (Plate I., Figs. 5–7), and goes undivided to one pole, thus constituting a heterochromosome group of the X-type, and in the late anaphase this group goes to one of the daughter plates (Fig. 7); thus forming two types of spermatocytes, one having eighteen and the other only twelve chromosomes. Polar views of the telophases of this division show that one plate receives half of the autosome material and all of the idiosome material, and that the other daughter plate receives only one half of the autosome material (Plate I., Figs. 8*a* and 8*b*). Sister second spermatocytes in the metaphase show very clearly the difference in the number of chromosomes found in the two types of second spermatocytes (Plate I., Fig. 9). Lateral views of the same stage show the size difference in the equatorial plate (Plate I., Fig. 10*a* with 18 chromosomes, Fig. 10*b* with 12 chromosomes).

The second division of the spermatocytes is entirely regular, each spermatid receiving one half the amount of chromatic material. In lateral views of the telophase of the second division the daughter plates from an eighteen chromosome second spermatocyte are larger than those from a twelve chromosome second spermatocyte (Plate I., Figs. 11*a* and 11*b*). Two types of spermatids can be recognized, those with larger nuclei and those with smaller nuclei. The chromosomes at this stage have become so diffused that they can no longer be distinguished as individual bodies (Plate I., Fig. 12*a*, eighteen chromosomes; Fig. 12*b*, twelve chromosomes).

2. *A. felis.*

Polar views of the metaphase plates of the first spermatocyte division show nine tetrad chromosomes, one of which is asymmetrical and larger than any of the remaining eight. This large tetrad is composed of two unequal parts, the larger component being as large as one of the ordinary tetrads. The smaller

component is about half the size of the autosome tetrads. The first division is transverse, and separates the unequal components of the large tetrad, or hexad, which is doubtlessly to be classed as a sex-chromosome, either of the XY-type or as an X-chromosome attached to the end of an autosome tetrad.

In the first division the one daughter plate receives eight diad autosomes and the larger component of the heterochromosome, and the other plate receives eight autosome diads and the smaller component of the heterochromosome. The second spermatocyte division is equational and entirely regular, each spermatid receiving nine chromosomes. As there are two types of second spermatocytes, there are also two types of spermatids formed, one having nine chromosomes all of the same size, and the other having nine chromosomes, one of which is larger than the other eight.

These results agree with those obtained by Edwards ('11) in his work on *A. felis*, and indicate that in all probability the form studied by the writer and by Edwards are the same form of *A. felis*. Since the figures made from my slides are in all respects similar to those published by Edwards, it was thought unnecessary to reproduce them here.

Discussion.

The present work shows *A. canis* different both morphologically and cytologically from *A. felis*. It shows also that the Nematodes worked upon by Marcus were evidently not *A. canis*. Marcus did not offer any taxonomic evidence that he was dealing with *A. canis*, this being merely another case in which a cytologist has not given proper consideration to the systematic standing of his material. The spermatocytes in the Nematodes described in Marcus's work contained twelve chromosomes, ten bivalents and two univalents, and no heterochromosome group. As errors in observation can not account for such diversity, it is doubtless due to differences in material.

Summary.

The following are the most important points brought out in this study:

1. Material used is same as recognized European forms of *Ascaris canis* (Werner) and *Ascaris felis* (Göze).

2. *A. canis* shows 18 chromosomes for the haploid number, 12 di-tetrad autosomes and 6 tetrad idiosomes. These 6 tetrad idiosomes form a heterosome group of the X-type.

3. There are two types of second spermatocytes and spermatids found, one type having 12 autosomes and 6 idiosomes, the other type having but the 12 autosomes.

4. In *A. felis* there are 9 chromosomes in the primary spermatocytes, 8 autosomes and one large heterochromosome composed of unequal parts. This is in agreement with Edwards.

5. *A. canis* and *A. felis* are morphologically and cytologically two different species, not varieties of the same species.

6. Chromosomes of true *A. canis* do not agree in form or number with those described by Marcus for his "so-called" *A. canis*.

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EXPLANATION OF PLATES.

All figures were made with the aid of a camera lucida, at a magnification of 2,800 diameters; Spencer apochromatic objective 2 mm., compensating ocular 20 × and projection distance 310 mm.

FIG. 1. Prophase of first spermatocyte, showing formation of chromosomes and separate grouping of heterochromosomes. The plasmosome is just above heterochromosome group.

FIG. 2. Late prophase, first spermatocyte, spindle formed but nuclear membrane still intact.

FIG. 3. Metaphase plate, first spermatocyte, showing heterochromosome group in center; number of chromosomes, 18.

FIG. 4. Metaphase plate, first spermatocyte, lateral view showing breaking down of nuclear membrane.

FIG. 5. Early anaphase, first spermatocyte, optical section through equatorial plate, showing lagging heterochromosome group.

FIG. 6. Late anaphase, first spermatocyte, showing lagging heterochromosome group of six components.

FIG. 7. Late anaphase, first spermatocyte, heterochromosome group going to upper daughter cell. Central spindle degenerated.

FIG. 8*a*. Polar view of daughter plate of first division receiving heterochromosome group; number of chromosomes, 18.

FIG. 8*b*. Polar view of daughter plate of first division not receiving heterochromosome group; number of chromosomes, 12.

FIG. 9. Metaphase plates of two sister second spermatocytes, which have not separated. One plate contains 18, the other 12 chromosomes.

FIG. 10*a*. Metaphase plate, second spermatocyte, lateral view of plate containing 18 chromosomes, *i. e.*, containing heterochromosome group of 6 and 12 autosomes.

FIG. 10*b*. Metaphase plate, second spermatocyte, lateral view of plate containing 12 chromosomes, *i. e.*, lacking the heterochromosome group of six.

FIG. 11*a*. Telophase of second spermatocyte division, having 18 chromosomes.

FIG. 11*b*. Telophase of second spermatocyte division, having 12 chromosomes.

FIG. 12*a*. Spermatid having 18 chromosomes.

FIG. 12*b*. Spermatid having 12 chromosomes.